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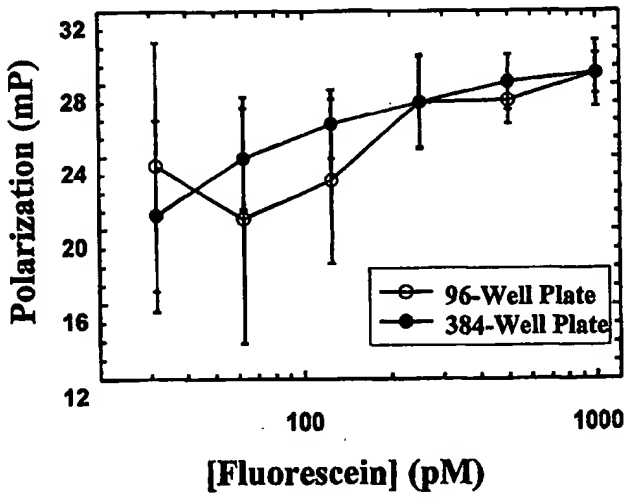

(57) Abstract

An apparatus for measuring polarized light emitted from a composition that includes a stage configured to hold a microplate having an array of sample wells, a continuous high color temperature light source, and a detector. An excitation optical relay structure directs light from the light source through an excitation polarizer toward a composition in one of the sample wells. An emission optical relay structure directs light emitted from the composition through an emission polarizer toward the detector.

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APPARATUS AND METHODS FOR MEASURING FLUORESCENCE POLARIZATION

Cross-References to Related Applications

This application is a continuation of the following patent applications, each of which is incorporated herein by reference: U.S. Patent Application Serial No. 09/062,472, filed April 17, 1998; U.S. Patent Application Serial No. 09/156,318, filed September 18, 1998; and U.S. Patent Application Serial No. 09/160,533, filed September 24, 1998.

This application is based upon and claims benefit under 35 U.S.C. § 119 of the following U.S. Provisional Patent Applications, each of which is incorporated herein by reference: Serial No. 60/063,811, filed October 31, 1997; Serial No. 60/072,499, filed January 26, 1998; Serial No. 60/072,780, filed January 27, 1998; Serial No. 60/075,414, filed February 20, 1998; Serial No. 60/075,806, filed February 24, 1998; Serial No. 60/082,253, filed April 17, 1998; Serial No. 60/084,167, filed May 4, 1998; Serial No. 60/085,335, filed May 13, 1998; Serial No. 60/085,500, filed May 14, 1998; Serial No. 60/089,848, filed June 19, 1998; Serial No. 60/092,203; Serial No. 60/094,275, filed July 27, 1998; Serial No. 60/094,276, filed July 27, 1998; Serial No. 60/100,817, filed September 18, 1998; Serial No. 60/100,951, filed September 18, 1998; and Serial No. 60/104,964, filed October 20, 1998.

Field of the Invention

The invention relates to photoluminescence polarization. More particularly, the invention relates to devices and methods for measuring photoluminescence polarization with enhanced sensitivity.

Background of the Invention

Fluorescence spectroscopic assays use fluorescence to characterize the components and properties of molecular systems; for example,

fluorescence assays may be used in high-throughput screening procedures to identify candidate drug compounds. Fluorescence spectroscopic assays may be based on fluorescence polarization (FP), fluorescence resonance energy transfer (FRET), fluorescence lifetime (FLT), total internal reflection (TIR) fluorescence, fluorescence correlation spectroscopy (FCS), and fluorescence recovery after photobleaching (FRAP), among others, with each approach having strengths and weaknesses. For example, fluorescence polarization assays are homogeneous and ratiometric, making them relatively insensitive to sample-to-sample variations in concentration, volume, or meniscus shape.

Fluorescence polarization assays involve the absorption and emission of polarized light. (Polarization describes the direction of light's electric field, which generally is perpendicular to the direction of light's propagation.) In a fluorescence polarization assay, a fluorescent sample is illuminated with polarized excitation light, which preferentially excites fluorophores having absorption dipoles aligned with the polarization of the excitation light. These molecules subsequently decay by preferentially emitting light polarized parallel to their emission dipoles. The extent to which the total emitted light is polarized depends on the extent of molecular reorientation during the lifetime of the excited state, which in turn depends on the size, shape, and environment of the reorienting molecule. Thus, fluorescence polarization assays may be used to quantify binding reactions and enzymatic activity, among other applications.

Fluorescence polarization assays have been conducted using various light sources. In academic research laboratories, light sources for fluorescence polarization assays have included lasers and arc lamps (e.g., xenon arc lamps). Unfortunately, these light sources suffer from a number of shortcomings. The gas in xenon arc lamps is under high pressure (about 10 atmospheres), so that explosion is always a danger. The power supplies for

lasers and xenon arc lamps operate at very high currents (about 25 amps) and voltages (about 20,000 to 40,000 volts), so that electrocution and other health hazards are always a danger. In particular, the power supplies for arc lamps produce ozone and may deliver a lethal shock when the lamps are started. The power supplies also may produce transients that can damage other electronic components of the system. The light emitted by lasers and xenon arc lamps is very intense, so that eye damage is always a danger. In particular, the extreme brightness may damage the retina, and ultraviolet light emitted by xenon arc lamps and some lasers may damage the cornea. The spectral output of lasers and some (e.g., mercury) arc lamps is very limited, so that desired excitation wavelengths may not be available. The lifetime of arc lamps may be very short, typically around 300 hours, so that the lamp must be changed frequently, further exposing the operator to dangers posed by the lamp and power supply.

These shortcomings assume even greater significance outside the research laboratory. For example, in high-throughput screening applications, the light source may be used nearly continuously, so that the dangers posed by lasers and arc lamps are ever present. The light source also may be used by relatively unskilled operators, who may be unfamiliar with or unreceptive to safety issues.

In high-throughput screening laboratories, light sources for fluorescence polarization assays have included incandescent (e.g., tungsten) lamps and flash lamps. Incandescent lamps are relatively common and inexpensive, and include lamps from overhead projectors. Incandescent lamps put out broad-spectrum light, so that they may be used with a variety of fluorescent compounds. Flash lamps are more exotic, but provide some advantages over incandescent lamps. In particular, flash lamps may be used for both time-resolved and steady-state measurements. This flexibility allows the same light source to be used in instruments that perform multiple assays, such

as steady-state and time-resolved fluorescence polarization assays. Moreover, flash lamps may have long lifetimes, as long as 10,000 hours.

Summary of the Invention

The invention provides a highly sensitive high throughput apparatus and method for measuring polarized light emitted from compositions. An apparatus employs a stage for supporting a container such as a microplate. A light source is connected to an excitation optical relay structure that directs light through an excitation polarizer toward a composition in the container at an examination site. An emission optical relay structure transmits light emitted from the composition through an emission polarizer, and then directs the polarized light toward a detector. The components of the apparatus are selected and configured so that photon noise is minimized relative to the magnitude of light emitted from small concentrations of fluorophore at the examination site.

In a preferred embodiment, the light source is a continuous high color temperature lamp such as a xenon arc lamp. The light source also may be a laser or a light emitting diode.

The functional elements of the apparatus may be contained in one or more rigid housings that are configured to minimize potential hazards associated with high temperature lamps.

The nature of the invention will be understood more readily after consideration of the drawings and the detailed description of the preferred embodiment that follow.

Brief Description of the Drawings

Figure 1 is a schematic view of fluorescently labeled molecules, showing how molecular reorientation affects fluorescence polarization.

Figure 2 is a schematic view of an apparatus for measuring fluorescence polarization in accordance with the invention.

Figure 3 is a schematic view of an alternative apparatus for measuring fluorescence polarization in accordance with the invention.

Figure 4 is a schematic partial perspective view of the apparatus of Figure 3.

Figure 5 is a schematic view of optical components from the apparatus of Figure 3.

Figure 6 is a partially exploded perspective view of a housing for the apparatus of Figure 3.

Figure 7 is a graph of polarization versus fluorescein concentration in 96-well and 384-well microplates, showing the sensitivity of the apparatus.

Figure 8 is a graph of the standard deviation of polarization versus fluorescein concentration in 384-well microplates, determined after 4-minute and 9-minute whole microplate read times, showing the sensitivity of the apparatus.

Figure 9 is a bar graph of polarization versus α-casein aggregation state, showing how fluorescence polarization may be used to measure binding equilibria.

Figure 10 is a graph of polarization versus time in solutions of keyhole-limpet haemocyanin with and without bovine pancreatic proteases, showing how fluorescence polarization may be used to measure enzyme activity.

Figure 11 is a graph of polarization versus phosphotyrosine competitor concentration in 96-well and 1536-well microplates, showing how fluorescence polarization may be used in a homogeneous immunoassay format to measure inhibition of enzyme activity.

Figure 12 is a graph of polarization versus phosphotyrosine competitor concentration in 384-well microplates, determined for sample volumes of 40, 60, 80, and 100 μL, showing how fluorescence polarization may be used in a homogeneous immunoassay format to measure inhibition of enzyme activity.

Detailed Description of the Invention

The invention provides devices and methods for measuring the polarization of light emitted from a composition. Generally, these devices and methods employ a light source, a detector, and an excitation and emission optical relay structure (having excitation and emission polarizers) for directing light between the light source, composition, and detector. For clarity, the description of the invention that follows is divided into six parts: (1) description of polarization, (2) description of polarization apparatuses, (3) methods of measuring polarization, (4) enhancement of signal, (5) description of preferred light sources, and (6) description of polarization experiments.

Overview of Polarization

Figure 1 is a schematic view showing how fluorescence polarization is affected by molecular reorientation. In a fluorescence polarization assay, specific molecules 30 within a composition 32 are labeled with one or more fluorophores. The composition then is illuminated with polarized excitation light, which preferentially excites fluorophores (shown in black) having absorption dipoles aligned parallel to the polarization of the excitation light. Excited fluorophores subsequently decay by preferentially emitting light polarized parallel to their emission dipoles.

The extent to which the total emitted light is polarized depends on the extent of molecular reorientation during the time interval between fluorescence excitation and emission, which is termed the fluorescence lifetime, τ_F. The extent of molecular reorientation in turn depends on the fluorescence

lifetime and the size, shape, and environment of the reorienting molecule. In particular, molecules rotate via diffusion with a rotational correlation time τ_{rot} that is proportional to their size. Thus, during the fluorescence lifetime, relatively large molecules will not reorient significantly, so that the total fluorescence will be relatively polarized. In contrast, during the same time interval, relatively small molecules will reorient significantly, so that the total fluorescence will be relatively unpolarized.

The relationship between polarization and intensity is expressed by the following equation:

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}} \qquad (1)$$

Here, P is the polarization, $I_{\parallel}$ is the intensity of fluorescence polarized parallel to the polarization of the excitation light, and $I_{\perp}$ is the intensity of fluorescence polarized perpendicular to the polarization of the excitation light. If there is little rotation between excitation and emission, $I_{\parallel}$ will be relatively large, $I_{\perp}$ will be relatively small, and P will be close to one. (P may be less than one even if there is no rotation; for example, P will be less than one if the absorption and emission dipoles are not parallel.) In contrast, if there is significant rotation between absorption and emission, $I_{\parallel}$ will be comparable to $I_{\perp}$, and P will be close to zero. Polarization often is reported in milli-P units (1000×P), which will range between 0 and 1000, because P will range between zero and one.

The relationship between polarization and rotation is expressed by the Perrin equation:

$$\left(\frac{1}{P} - \frac{1}{3}\right) = \left(\frac{1}{P_0} - \frac{1}{3}\right) \cdot \left(1 + \frac{\tau_F}{\tau_{rot}}\right) \qquad (2)$$

Here, P_0 is the polarization in the absence of molecular motion (intrinsic polarization), τ_F is the fluorescence lifetime (inverse decay rate) as described

above, and τ_{rot} is the rotational correlation time (inverse rotational rate) as described above.

The Perrin equation shows that fluorescence polarization assays are most sensitive when the fluorescence lifetime and the rotational correlation time are similar. Rotational correlation time is proportional to molecular weight, increasing by about 1 nanosecond for each 2,400 dalton increase in molecular weight (for a spherical molecule). For shorter lifetime fluorophores, such as fluorescein, which has a fluorescence lifetime of roughly 4 nanoseconds, fluorescence polarization assays are most sensitive for molecular weights less than about 40,000 daltons. For longer lifetime probes, such as $Ru(bpy)_2dcbpy$ (ruthenium 2,2'-dibipyridyl 4,4'-dicarboxyl-2,2'-bipyridine), which has a lifetime of roughly 400 nanoseconds, fluorescence polarization assays are most sensitive for molecular weights between about 70,000 daltons and 4,000,000 daltons.

Description of Polarization Apparatuses

Figure 2 is a schematic view of an apparatus 50 for measuring fluorescence polarization. Apparatus 50 includes a light source 52, an excitation polarizer 54, an emission polarizer 56, and a detector 58. Light 60 produced by light source 52 is directed through excitation polarizer 54, which passes polarized excitation light (indicated by vertical arrow). Polarized excitation light is directed onto a fluorescent sample 62, which produces fluorescence emission light 64 in response. Fluorescence emission light 64 is directed through emission polarizer 56, which may have components oriented parallel ($\|$; indicated by vertical arrow) or perpendicular ($\perp$; indicated by horizontal arrow) to the polarization of excitation light 60. Depending on its orientation, emission polarizer 56 passes parallel ($I_\|$) or perpendicular ($I_\perp$) components of emission light 64 for detection by detector 58.

Figures 3-6 are schematic views of an alternative apparatus 90 for measuring fluorescence polarization. Apparatus 90 may include one or more light sources for delivering light to a composition, one or more detectors for receiving light transmitted from the composition, and an optical relay structure for relaying light between the light source, composition, and detector. Apparatus 90 may be used for a variety of fluorescence and absorbance assays.

As configured here, apparatus 90 includes a continuous light source 100 and a time-modulated light source 102. Apparatus 90 includes light source slots 103a-d for four light sources, although other numbers of light source slots and light sources also could be provided. Light source slots 103a-d function as housings that may surround at least a portion of each light source, providing some protection from radiation and explosion. The direction of light transmission through the photoluminescence optical system is indicated by arrows.

Continuous source 100 provides light for photoluminescence intensity and steady-state photoluminescence polarization assays. Continuous light souce 100 may include arc lamps, lasers, and light-emitting diodes, among others. A preferred continuous source is a high-intensity, high color temperature xenon arc lamp, such as a Model LX175F CERMAX xenon lamp from ILC Technology, Inc. Color temperature is the absolute temperature in kelvins at which a blackbody radiator must be operated to have a chromaticity equal to that of the light source. A high color temperature lamp produces more light than a low color temperature lamp, and it may have a maximum output shifted toward or into visible wavelengths and ultraviolet wavelengths where many fluorophores absorb. The preferred continuous source has a color temperature of 5600 Kelvin, greatly exceeding the color temperature of about 3000 Kelvin for a tungster filament source. The preferred source provides more light per unit time than flash sources, increasing sensitivity and reducing read

times. Apparatus 90 may include a modulator mechanism configured to vary the intensity of light incident on the composition without varying the intensity of light produced by the light source.

Time-modulated source 102 provides light for time-resolved photoluminescence assays, such as photoluminescence lifetime and time-resolved photoluminescence polarization assays. A preferred time-modulated source is a xenon flash lamp, such as a Model FX-1160 xenon flash lamp from EG&G Electro-Optics. The preferred source produces a "flash" of light for a brief interval before signal detection and is especially well suited for time-domain measurements. Other time-modulated sources include pulsed lasers, as well as continuous lamps whose intensity can be modulated extrinsically using a Pockels cell, Kerr cell, or other mechanism. The latter sources are especially well suited for frequency-domain measurements.

In apparatus 90, continuous source 100 and time-modulated source 102 produce multichromatic, unpolarized, and incoherent light. Continuous source 100 produces substantially continuous illumination, whereas time-modulated source 102 produces time-modulated illumination. Light from these light sources may be delivered to the sample without modification, or it may be filtered to alter its intensity, spectrum, polarization, or other properties.

Light produced by the light sources follows an excitation optical path to an examination site. Such light may pass through one or more "spectral filters," which generally comprise any mechanism for altering the spectrum of light that is delivered to the sample. Spectrum refers to the wavelength composition of light. A spectral filter may be used to convert white or multichromatic light, which includes light of many colors, into red, blue, green, or other substantially monochromatic light, which includes light of one or only a few colors. In apparatus 90, spectrum is altered by an excitation interference filter 104, which selectively transmits light of preselected wavelengths and

selectively absorbs light of other wavelengths. For convenience, excitation interference filters 104 may be housed in an excitation filter wheel 106, which allows the spectrum of excitation light to be changed by rotating a preselected filter into the optical path. Spectral filters also may separate light spatially by wavelength. Examples include gratings, monochromators, and prisms.

Spectral filters are not required for monochromatic ("single color") light sources, such as certain lasers, which output light of only a single wavelength. Therefore, excitation filter wheel 106 may be mounted in the optical path of some light source slots 103a,b, but not other light source slots 103c,d.

Light next passes through an excitation optical shuttle (or switch) 108, which positions an excitation fiber optic cable 110a,b in front of the appropriate light source to deliver light to top or bottom optics heads 112a,b, respectively. The optics heads include various optics for delivering light into the sensed volume and for receiving light transmitted from the sensed volume. Light is transmitted through a fiber optic cable much like water is transmitted through a garden hose. Fiber optic cables can be used easily to turn light around corners and to route light around opaque components of the apparatus. Moreover, fiber optic cables give the light a more uniform intensity profile. A preferred fiber optic cable is a fused silicon bundle, which has low autofluorescence. Despite these advantages, light also can be delivered to the optics heads using other mechanisms, such as mirrors.

Light arriving at the optics head may pass through one or more excitation "polarization filters," which generally comprise any mechanism for altering the polarization of light. Excitation polarization filters may be included with the top and/or bottom optics head. In apparatus 90, polarization is altered by excitation polarizers 114, which are included only with top optics head 112a. Excitation polarization filters 114 may include an s-polarizer S that passes only

s-polarized light, a p-polarizer P that passes only p-polarized light, and a blank O that passes substantially all light. Excitation polarizers 114 also may include a standard or ferro-electric liquid crystal display (LCD) polarization switching system. Such a system is faster and more economical than a mechanical switcher. Excitation polarizers 114 also may include a continuous mode LCD polarization rotator with synchronous detection to increase the signal-to-noise ratio in polarization assays.

Light at one or both optics heads also may pass through an excitation "confocal optics element," which generally comprises any mechanism for focusing light into a "sensed volume." In apparatus 90, the confocal optics element includes a set of lenses 117a-c and an excitation aperture 116 placed in an image plane conjugate to the sensed volume, as shown in Figure 5. Aperture 116 may be implemented directly, as an aperture, or indirectly, as the end of a fiber optic cable. Lenses 117a,b project an image of aperture 116 onto the sample, so that only a preselected or sensed volume of the sample is illuminated.

Light traveling through the optics heads is reflected and transmitted through a beamsplitter 118, which delivers reflected light to a composition 120 and transmitted light to a light monitor 122. Reflected and transmitted light both pass through lens 117b, which is operatively positioned between beamsplitter 118 and composition 120.

The beamsplitter is used to direct excitation light toward the sample and light monitor, and to direct emission light toward the detector. The beamsplitter is changeable, so that it may be optimized for different assay modes or compositions. If a large number or variety of luminescent molecules are to be studied, the beamsplitter must be able to accommodate light of many wavelengths; in this case, a "50:50" beamsplitter that reflects half and transmits half of the incident light independent of wavelength is optimal. Such

a beamsplitter can be used with many types of molecules, while still delivering considerable excitation light onto the composition, and while still transmitting considerable emission light to the detector. If one or a few related luminescent molecules are to be studied, the beamsplitter needs only to be able to accommodate light at a limited number of wavelengths; in this case, a "dichroic" or "multichroic" beamsplitter is optimal. Such a beamsplitter can be designed with cutoff wavelengths for the appropriate set of molecules and will reflect most or substantially all of the excitation and background light, while transmitting most or substantially all of the emission light. This is possible because the reflectivity and transmissivity of the beamsplitter can be varied with wavelength.

The light monitor is used to correct for fluctuations in the intensity of light provided by the light sources; such corrections are performed by reporting detected intensities as a ratio over corresponding times of the luminescence intensity measured by the detector to the excitation light intensity measured by the light monitor. The light monitor also can be programmed to alert the user if the light source fails. A preferred light monitor is a silicon photodiode with a quartz window for low autofluorescence.

The composition (or sample) is held in a sample holder supported by a stage 123. The composition can include compounds, mixtures, surfaces, solutions, emulsions, suspensions, cell cultures, fermentation cultures, cells, tissues, secretions, and/or derivatives and/or extracts thereof. Analysis of the composition may involve measuring the presence, concentration, or physical properties (including interactions) of a photoluminescent analyte in such a composition. The sample holder can include microplates, biochip, or any array of samples in a known format. In apparatus 90, the preferred sample holder is a microplate 124, which includes a plurality of microplate wells 126 for holding

compositions. Composition may refer to the contents of a single microplate well, or several microplate wells, depending on the assay.

The sensed volume typically has an hourglass shape, with a cone angle of about 25° and a minimum diameter ranging between 0.1 mm and 2.0 mm. For 96-well and 384-well microplates, a preferred minimum diameter is about 1.5 mm. For 1536-well microplates, a preferred minimum diameter is about 1.0 mm. The size and shape of the sample container may be matched to the size and shape of the sensed volume.

The position of the sensed volume can be moved precisely within the composition to optimize the signal-to-noise and signal-to-background ratios. For example, the sensed volume may be moved away from walls in the sample holder to optimize signal-to-noise and signal-to-background ratios, reducing spurious signals that might arise from fluorophore bound to the walls and thereby immobilized. In apparatus 90, position in the X,Y-plane perpendicular to the optical path is controlled by moving the stage supporting the composition, whereas position along the Z-axis parallel to the optical path is controlled by moving the optics heads using a Z-axis adjustment mechanism 130, as shown in Figures 3 and 4. However, any mechanism for bringing the sensed volume into register or alignment with the appropriate portion of the composition also may be employed.

The combination of top and bottom optics permits assays to combine: (1) top illumination and top detection, or (2) top illumination and bottom detection, or (3) bottom illumination and top detection, or (4) bottom illumination and bottom detection. Same-side illumination and detection (1) and (4) is referred to as "epi" and is preferred for photoluminescence assays. Opposite-side illumination and detection (2) and (3) is referred to as "trans" and is preferred for absorbance assays. In apparatus 90, epi modes are supported, so the excitation and emission light travel the same path in the optics

head, albeit in opposite or anti-parallel directions. However, trans modes also could be supported and would be essential for absorbance assays. Generally, top optics can be used with any sample holder having an open top, whereas bottom optics can be used only with sample holders having optically transparent bottoms, such as glass or thin plastic bottoms.

Light is transmitted by the composition in multiple directions. A portion of the transmitted light will follow an emission pathway to a detector. Transmitted light passes through lens 117c and may pass through an emission aperture 131 and/or an emission polarizer 132. In apparatus 90, the emission aperture is placed in an image plane conjugate to the sensed volume and transmits light substantially exclusively from this sensed volume. In apparatus 90, the emission apertures in the top and bottom optical systems are the same size as the associated excitation apertures, although other sizes also may be used. The emission polarizers are included only with top optics head 112a. The emission aperture and emission polarizer are substantially similar to their excitation counterparts.

Excitation polarizers 114 and emission polarizers 132 may be used together in nonpolarization assays to reject certain background signals. Luminescence from the sample holder and from luminescent molecules adhered to the sample holder is expected to be polarized, because the rotational mobility of these molecules should be hindered. Such polarized background signals can be eliminated by "crossing" the excitation and emission polarizers, that is, setting the angle between their transmission axes at 90°. As described above, such polarized background signals also can be reduced by moving the sensed volume away from walls of the sample holder. To increase signal level, beamsplitter 118 should be optimized for reflection of one polarization and transmission of the other polarization. This method will work best where the

luminescent molecules of interest emit relatively unpolarized light, as will be true for small luminescent molecules in solution.

Transmitted light next passes through an emission fiber optic cable 134a,b to an emission optical shuttle (or switch) 136. This shuttle positions the appropriate emission fiber optic cable in front of the appropriate detector. In apparatus 90, these components are substantially similar to their excitation counterparts, although other mechanisms also could be employed.

Light exiting the fiber optic cable next may pass through one or more emission "intensity filters," which generally comprise any mechanism for reducing the intensity of light. Intensity refers to the amount of light per unit area per unit time. In apparatus 90, intensity is altered by emission neutral density filters 138, which absorb light substantially independent of its wavelength, dissipating the absorbed energy as heat. Emission neutral density filters 138 may include a high-density filter H that absorbs most incident light, a medium-density filter M that absorbs somewhat less incident light, and a blank O that absorbs substantially no incident light. These filters are changed by hand, although other methods also could be employed, such as a filter wheel. Intensity filters also may divert a portion of the light away from the sample without absorption. Examples include beam splitters, which transmit some light along one path and reflect other light along another path, and Pockels cells, which deflect light along different paths through diffraction.

Light next may pass through an emission interference filter 140, which may be housed in an emission filter wheel 142. In apparatus 90, these components are substantially similar to their excitation counterparts, although other mechanisms also could be employed. Emission interference filters block stray excitation light, which may enter the emission path through various mechanisms, including reflection and scattering. If unblocked, such stray excitation light could be detected and misidentified as photoluminescence,

decreasing the signal-to-background ratio. Emission interference filters can separate photoluminescence from excitation light because photoluminescence has longer wavelengths than the associated excitation light.

The relative positions of the spectral, intensity, polarization, and other filters presented in this description may be varied without departing from the spirit of the invention. For example, filters used here in only one optical path, such as intensity filters, also may be used in other optical paths. In addition, filters used here in only top or bottom optics, such as polarization filters, may also be used in the other of top or bottom optics or in both top and bottom optics. The optimal positions and combinations of filters for a particular experiment will depend on the assay mode and the composition, among other factors.

Light last passes to a detector, which is used in absorbance and photoluminescence assays. In apparatus 90, there is one photoluminescence detector 144, which detects light from all photoluminescence modes. A preferred detector is a photomultiplier tube (PMT). Apparatus 90 includes detector slots 145a-d for four detectors, although other numbers of detector slots and detectors also could be provided.

More generally, detectors comprise any mechanism capable of converting energy from detected light into signals that may be processed by the apparatus. Suitable detectors include photomultiplier tubes, photodiodes, avalanche photodiodes, charge-coupled devices (CCDs), and intensified CCDs, among others. Depending on the detector and assay mode, such detectors may be used in (1) photon-counting or continuous modes, and (2) imaging or integrating modes.

Figure 6 is a partially exploded perspective view of a housing 150 and other accessories for the apparatus of Figures 3-5. Housing 150 substantially encloses the apparatus, forming (together with light source slots

103a-d) two protective layers around the continuous high color temperature xenon arc lamp. Housing 150 permits automated sample loading and switching among light sources and detectors, further protecting the operator from the xenon arc lamp.

Methods of Measuring Polarization

Apparatus 90 may be used to conduct steady-state and time-resolved polarization assays. Steady-state polarization assays measure polarization under constant illumination, using the continuous light source. Time-resolved polarization assays measure polarization as a function of time, using the time-varying light source.

Steady-state polarization assays may be conducted as follows. Excitation light from the continuous light source is directed through an excitation filter, low-luminescence fiber optic cable, and excitation polarization filter. Excitation light then is directed to a beamsplitter, which reflects most of the light onto a composition and transmits a little of the light into a light monitor. Emitted light from the composition is directed back through the beamsplitter and then is directed through another low-luminescence fiber optic cable, an emission filter, and a polarization filter (in either the S or P orientation) before detection by a photomultiplier tube.

Two measurements are performed for each composition, one with excitation and emission polarizers aligned and one with excitation and emission polarizers crossed. Either polarizer may be static or dynamic, and either polarizer may be set in the S or P orientation, although typically the excitation polarizer is set in the S orientation.

Steady-state polarization assays also may be conducted by constantly polarizing and transmitting high color temperature light to an examination site as successive samples are automatically, serially aligned in an

optical path intersecting the examination site, and detecting polarized light emitted from each sample.

Enhancement of Signal

Achieving good signal-to-noise and signal-to-background ratios from dilute samples is critical in fluorescence polarization and other assays supported by the apparatus. For example, in a binding assay, it often is desirable to be able to probe binding involving molecules with dissociation constants in the sub-nanomolar range. This is facilitated by being able to achieve acceptable signal-to-noise and signal-to-background ratios from compositions with fluorophore concentrations in the sub-nanomolar range. The methods of signal-to-noise and signal-to-background enhancement discussed below permit the apparatus to achieve the required sensitivity with such dilute samples, thereby minimizing reagent cost, which otherwise can be considerable.

Generally, components of the apparatus are chosen to enhance sensitivity and dynamic range, both in fluorescence polarization assays and in other assays supported by the apparatus. Toward this end, optical components with low intrinsic luminescence and high intrinsic throughput are chosen. In addition, some components are shared by different modes, whereas other components are unique to a particular mode. For example, photoluminescence intensity and steady-state photoluminescence polarization modes share a continuous light source; time-resolved luminescence modes share a time-varying light source, and chemiluminescence modes do not use a light source. Similarly, photoluminescence and chemiluminescence modes use different detectors.

Sensitivity also is enhanced by reducing the contribution of noise to the measurements. In fluorescence polarization assays, various factors contribute to noise, including (1) background noise and (2) intensity noise.

Background noise refers to contributions to the signal from fluorescent species other than the fluorescent species of interest, including fluorescent species in the apparatus and sample holder. Intensity noise refers to fluctuations in light intensity, including those arising from photon noise.

Background noise may be reduced by reducing autofluorescence from the apparatus and sample holder. For example, the apparatus may use low luminescence components, such as fused silica fiber optic cables. Similarly, the sample holder may be constructed of low luminescence materials, such as black polystyrene.

Background noise also may be reduced by reducing detection of fluorescence from components of the sample that are bound to the sample holder and immobilized, which otherwise would lead to spuriously high fluorescence polarization. For example, the walls of the sample holder may be constructed or treated to reduce binding. Alternatively, in an apparatus capable of detecting light transmitted substantially exclusively from a sensed volume, the sensed volume may be positioned near the center of the composition, away from the walls of the sample holder.

Intensity noise may be reduced by correcting for fluctuations in light source intensity, among others. Light source fluctuations arise due to fluctuations in power from the power supply and drift in the position of the arc in arc lamps, among others. Light source fluctuations will lead to fluorescence fluctuations, because the amount of fluorescence is proportional to the amount of excitation light. Fluorescence fluctuations are especially important in fluorescence polarization assays, because such assays involve comparing the magnitude of successively measured fluorescence signals. Light source fluctuations may be reduced by choosing a stable light source and by rescaling the fluorescence signal using information obtained from a light source monitor, as described above.

Intensity noise also may be reduced by increasing the number of photons (amount of light) detected, which reduces photon noise. Photon (or shot) noise arises due to the statistical nature of light and may be described by the same statistical law used to describe radiation decay. In particular, if N photons are detected during a given time interval, the standard deviation associated with that number due to photon noise will be $\sqrt{N}$. The relative significance of photon noise decreases as the number of detected photons increases, because the ratio of the standard deviation in the signal to the signal goes as $\sqrt{N}/N = 1/\sqrt{N}$. Although there may be many sources of intensity noise, the limit set by photon noise can never be overcome; however, the significance of photon noise can be reduced by increasing the number of photons collected by the detector. The number of photons collected may be increased by increasing the intensity of the light source, the efficiency of the detector, and/or the throughput of components of the optical relay structure, such as the beamsplitter, among others.

Photon noise creates noise in fluorescence polarization assays. To a very good approximation, the noise in the polarization is proportional to the noise in the fluorescence intensities from which the polarization is calculated and corresponds to seven mP standard deviation in polarization for every one percent standard deviation in intensity. This relationship essentially is independent of the degree of polarization. Because of photon noise, the requirement for rapid high-throughput screening measurements in the optically restrictive microplate format puts a premium on simply collecting enough light. For additional information, see the calculation in U.S. Provisional Patent Application Serial No. 60/063,811, which is incorporated herein by reference.

Most well-developed polarization assays have maximum polarization changes of between 100 mP and 200 mP, so acceptable standard

deviations in the polarization should be no greater than about 5 mP to 10 mP. This requires detection of at least 10,000 photons per intensity measurement to reduce intensity noise to about 1%. The inefficiency of polarization optical systems increases the problem. The number of photons collected is proportional to both the concentration and the detection time, leading to trade-offs between probe concentration and screening throughput. High concentrations of reagents not only are expensive, but also produce insensitive binding assays if they exceed the dissociation constant of the binding reaction.

Figures 7-8 show results that characterize a fluorescence polarization apparatus constructed in accordance with the invention. Data were collected at room temperature using the preferred apparatus shown in Figures 3-6.

Figure 7 is a graph showing polarization in a serial dilution of fluorescein in 96- and 384-well microplates. The graph demonstrates that the polarization of fluorescein can be measured with adequate accuracy and precision down to, or below, 100 pM, because the measured value is substantially independent of concentration down to, or below, this concentration.

Figure 8 is a graph showing the noise (standard deviation) in polarization in a serial dilution of fluorescein in 384-well microplates. Noise below 5-10 mP is sufficiently small for most practical FP assays, as described above. Good precision is obtained at sub-nanomolar label concentrations in rapidly scanned 384-well microplates. Even better precision is obtained in more slowly scanned microplates. The size of the error bars shows that the number of photons collected by the detector exceeds 10,000 in 100 milliseconds from a 100 picomolar fluorescein solution at pH 7.5.

Description of Preferred Light Sources

As described above, photon noise problems may be eliminated by using a sufficiently high-intensity light source, such as a continuous high color temperature xenon arc lamp or laser, among others. The following table compares the preferred continuous and time-varying light sources used in the apparatus disclosed in Figures 3-6.

Summary	Continuous Light Source	Flash Lamp Light Source	Comparison (Flash / Continuous)
Life of light source	300 hrs	10,000 hrs	6%
Total power of light source	13,000 mW	830 mW	5%
Visible power (390-770 nm)	5100 mW	230 mW	3%
Infrared power (>770 nm)	7300 mW	190 mW	11%
Ultraviolet power (300-390 nm)	620 mW	68 mW	4%
Apparatus power (485 nm)	7.1 mW 1.7×10^{16} photons/sec	0.29 mW 7.1×10^{14} photons/sec	4%
Photons/sec from 1 nM fluorophore solution (estimated)	1×10^{8} photons/sec	5×10^{6} photons/sec	5%
Photons/sec from 10 pM fluorophore solution (estimated)	1×10^{6} photons/sec	5×10^{4} photons/sec	5%

The continuous lamp has only 1/33 the lifetime of the flash lamp. The lifetime of the continuous lamp was taken directly from the manufacturer's specifications. The lifetime of the flash lamp was computed using the manufacturer's specification as follows. The flash lamp is run at 100 flashes per second, using 250 millijoules of electrical power per pulse. The flash lamp life

is rated for 1×10^9 - 1×10^{10} flashes at this power level, which translates to a lifetime of about 10,000 hours [5×10^9/(100 Hz × 3600 sec/hour)].

The continuous lamp provides about 20 times more light than the flash lamp. The total optical power of the continuous lamp (collected by a F/1.0 optical system) is 13 W over the range of 300-4000 nm. The total optical power of the flash lamp (collected by a F/1.0 optical system) is 830 mW over the range 100-4000 nm. The total flash lamp power was derived from the electrical energy, the electrical-to-optical conversion efficiency, the optical collection efficiency, and the repetition rate (250 mJ × 50% × 6.6% × 100 Hz). The optical power of the different spectra of the flash lamp was derived by multiplying the total flash lamp power by the fraction of the power in each wavelength range, i.e., visible (390-770 nm) 28%, infrared (770+ nm) 24%, and ultraviolet (300-390 nm) 8.3%.

The optical power in the preferred apparatus was determined after passage through a bandpass filter (center 485 nm, bandwidth 20 nm). The optical power in photons per second was calculated by assuming that all photons had a wavelength of 485 nm (energy = 1240eV × nm/wavelength).

High-throughput screening requires that light be collected quickly and effectively, so that assays may be accurately and rapidly performed. Obtaining a 1% error in intensity measurement, corresponding to a 7 mP error in polarization, requires collection of at least 10,000 photons, as described above. For high-throughput screening, these photons should be collected within 100 ms, corresponding to a collection rate of 100,000 photons / sec.

Both lamps produce more than 100,000 photons / per second, but the criterion is to collect 100,000 fluorescence photons / sec, not to produce that number of excitation photons. Specifically, the criterion is to count at least 10,000 photons in 100 ms (1×10^5 photon/s) for low concentrations of fluorophore (less than 1 nM). The preferred apparatus achieves this photon

limit at roughly 10-100 pM for FP assays. The sample holder provides an optical path of about 5 mm. Typical molar extinction coefficients are 50,000 per molar per centimeter. Based on the Beer-Lambert law [$\log(I_o/I)=\varepsilon cl$], the fraction of photons absorbed for a 1 nM solution is about 6×10^{-5} (about 6×10^{-7} for 10 pM). The quantum yield (ratio of photons emitted to photons absorbed) of typical fluorophores is 0.9, so that about 5×10^{-5} of the incoming photons are converted to fluorescence emission photons (at 1 nM). This is effectively the emission efficiency.

The collection efficiency of the apparatus also is important. Fluorescence is emitted in all directions, whereas fluorescence is collected over limited angles. The cone angle over which fluorescence is collected is given by the formula: $\theta = \arcsin[(NA)/n)]$, where NA is the numerical aperture and n is the index of refraction. The optical collection efficiency is 3% for an NA of 0.39, and it is 1% for an NA of 0.22. The light must also be transmitted to the detector. The transmission efficiency of the preferred apparatus probably is about 2%. Additionally, the detector has a quantum efficiency of detection, which typically is 20-25% for a PMT like that used in the preferred apparatus. The net result obtained after passing through all of these steps is the detection efficiency, which is given by the product of the emission efficiency, collection efficiency, transmission efficiency, and quantum efficiency, as follows:

Concentration	Detection Efficiency (Estimated)
1 nM	$5\times10^{-5} \times 0.03 \times 0.02 \times 0.25 = 8\times10^{-9}$
10 pM	$5\times10^{-7} \times 0.03 \times 0.02 \times 0.25 = 8\times10^{-11}$

The detection efficiency was multiplied by the excitation flux for the estimated measurable flux at 1 nM and 10 pM (measured in photons / sec). The flash lamp has enough optical power to make statistically significant

measurements at 1 nM, but not at 10 pM, where it has less than 1×10^5 photons/sec.

Taking into account the detection efficiency, the continuous lamp satisfies the collection criteria, whereas the flash lamp does not. Specifically, the continuous lamp fails the criterion of 100,000 photons per second somewhere below 10 pM for a typical fluorophore, whereas the flash lamp fails the criterion somewhere near 200 pM (roughly 20 times higher).

The continuous lamp has a power of greater than 1 watt over the visible wavelength range of 390 to 770 nm, and is sufficient to reduce photon noise to less than 1 percent of a light signal emitted from a 100 picomolar fluorescein solution at pH 7.5.

Description of Polarization Experiments

Figures 9-12 show applications of the invention to biological problems. Data were collected at room temperature using the preferred apparatus shown in Figures 3-6. Figures 9-12 show several applications of fluorescence polarization assays, including (1) measuring binding equilibria, (2) measuring enzyme activity, and (3) screening for inhibitors of enzyme activity.

Figure 9 shows fluorescence polarization in use to measure binding equilibria. Specifically, fluorescence polarization was used to monitor the aggregation of α-casein (MW 25,000 daltons), a protein from bovine milk. The casein was labeled with the NHS ester of $Ru(bpy)_2dcbpy$ (ruthenium 2,2'-dibipyridyl 4,4'-dicarboxyl-2,2'-bipyridine); free $Ru(bpy)_2dcbpy$ has a fluorescence lifetime (τ_F) of approximately 370 nanoseconds, an intrinsic polarization (P_0) of approximately 300 mP, and a combined extinction coefficient and quantum yield about 1% that of fluorescein. The protein was labeled at a ratio of 25 μg $Ru(bpy)_2dcbpy$ / mg protein, and the labeled protein was diluted in 50 mM TRIS, pH 7.8 to a final concentration of 100 μg/mL. Casein is highly negatively charged, with many phosphorylated groups. Casein

also is acidic, with an isoelectric pH of 4 to 4.5. In the absence of calcium, casein is completely deprotonated and in monomeric form. In the presence of calcium (e.g., 10 mM $CaCl_2$), casein is aggregated due to ionic bridges between individual proteins. As shown in Figure 9, monomeric protein has a low but measurable polarization of about 48 mP, whereas aggregated protein has a high polarization of about 200 mP.

More generally, fluorescence polarization assays for binding equilibria may be used to screen for inhibitors of ligand/receptor interactions by placing a labeled ligand in a system with its receptor and a potential inhibitor. Inhibitors will release labeled ligand that is bound to the receptor, changing the polarization, because bound ligand will have a high molecular weight and hence high polarization, whereas free ligand will have a low molecular weight and hence low polarization. Because the polarization of a mixture of bound and free molecules is related to the polarizations of the individual species, the amount of binding can be determined from a standard curve, without separating bound and free molecules. This technique also may be used to study cellular aggregation.

Figure 10 shows fluorescence polarization in use to detect enzyme activity. Specifically, fluorescence polarization was used to monitor the action of a mixture of bovine pancreatic proteases on keyhole-limpet haemocyanin. The haemocyanin was labeled as above, at a ratio of 100 μg $Ru(bpy)_2dcbpy$ / mg protein, and the labeled protein was diluted in 50 mM TRIS, pH 7.8 to a final concentration of 22 μg/mL. Bovine crude protease was added to the diluted labeled protein, to a final concentration of 5 μg/ml solution. As shown in Figure 10, the polarization fell from about 220 mP to about 30 mP as the protease cleaved the labeled protein to form smaller, more rapidly rotating fragments.

More generally, fluorescence polarization may be used to screen for enzyme activity itself, and for inhibitors of enzyme activity. Suitable enzymes include proteases and nucleases. Proteases are especially interesting because they are important in understanding and treating inflammatory disorders, infectious diseases, and apoptosis, among others.

Figures 11-12 show fluorescence polarization in use in a homogeneous immunoassay format to screen for modulators (usually inhibitors) of enzymes. Specifically, fluorescence polarization was used to monitor tyrosine kinase activity. Anti-phosphotyrosine antibody (1:750 dilution of ascites fluid) and fluorescein-labeled phosphotyramine (1 nM) were incubated with the indicated concentrations of phosphorylated tyrosine-kinase substrate (i.e., the enzyme product). The enzyme product competitively released the labeled compound from the antibody, decreasing the fluorescence polarization, because the polarization of the label is high when bound to the antibody and low when displaced from the antibody by the enzyme product. Thus, the higher the enzyme activity, the lower the fluorescence polarization.

Figures 11-12 show results obtained under a variety of assay conditions. Figure 11 shows results for 200 μL samples in 96-well microplates and 5 μL samples in 1536-well microplates. Error bars, averaging 4 to 8 mP, are standard deviations. The phosphotyrosine IC_{50} values determined from the data are 379 ± 22 nM for the 200 μL samples and 326 ± 30 nM for the 5 μL samples. Error bars are standard errors of the mean. Figure 12 shows results for 40, 60, 80, and 100 μL samples in 384-well microplates. Error bars, averaging 1 to 4 mP, are standard deviations. Error bars for polarizations in Figures 11-12 should be compared with the maximum change in polarization for the assay, which is about 110 mP.

Although the invention has been disclosed in its preferred form, the specific embodiments thereof as disclosed and illustrated herein are not to

be considered in a limiting sense, because numerous variations are possible. For example, although the invention is described primarily in terms of fluorescence polarization assays, the invention also covers phosphorescence polarization assays, which work by similar principles, the excited state decay coming from a triplet state rather than a singlet state. Applicants regard the subject matter of their invention to include all novel and non-obvious combinations and subcombinations of the various elements, features, functions, and/or properties disclosed herein. No single feature, function, element or property of the disclosed embodiments is essential. The following claims define certain combinations and subcombinations of features, functions, elements, and/or properties that are regarded as novel and non-obvious. Other combinations and subcombinations may be claimed through amendment of the present claims or presentation of new claims in this or a related application. Such claims, whether they are broader, narrower, or equal in scope to the original claims, also are regarded as included within the subject matter of applicants' invention.

WE CLAIM:

1. An apparatus for measuring the polarization of light emitted from a composition, the apparatus comprising:

a stage configured to hold a microplate having an array of sample wells;

a continuous high color temperature light source;

an excitation optical relay structure having an excitation polarizer, wherein the excitation optical relay structure directs light from the light source through the excitation polarizer toward a composition contained in at least one of the sample wells;

a detector; and

an emission optical relay structure having an emission polarizer, wherein the emission optical relay structure directs light emitted from the composition through the emission polarizer toward the detector.

2. The apparatus of claim 1, wherein the continuous high color temperature light source has a color temperature of at least about 3500 Kelvin.

3. The apparatus of claim 1, wherein the continuous high color temperature light source is a xenon arc lamp.

4. The apparatus of claim 1, further comprising a housing that surrounds at least a portion of the light source.

5. The apparatus of claim 1, wherein the apparatus is configured to perform luminescence polarization assays and at least one of the following additional assays: luminescence intensity, chemiluminescence, photoluminescence lifetime, absorbance, luminescence resonance energy transfer, and luminescence imaging.

6. The apparatus of claim 5, wherein the excitation polarizer and the emission polarizer are crossed to reduce background from immobilized species in the additional assays.

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7. The apparatus of claim 1, wherein adjacent the composition light directed toward the detector by the emission optical relay structure travels anti-parallel to light directed toward the composition by the excitation optical relay structure.

8. The apparatus of claim 1, wherein the apparatus is capable of detecting light transmitted substantially exclusively from a sensed volume of the composition.

20 9. The apparatus of claim 8, the composition being contained in a spatial volume lying between boundary interfaces, wherein the sensed volume is spaced substantially away from at least one of the boundary interfaces.

10. The apparatus of claim 1, further comprising a second light source, wherein the high color temperature light source is configured for steady-state polarization assays, and wherein the second light source is configured for time-resolved polarization assays.

11. The apparatus of claim 1, wherein the excitation and emission optical relay structures each include a fiber optic cable.

12. The apparatus of claim 1, wherein the excitation and emission optical relay structures share a dichroic beamsplitter.

13. The apparatus of claim 12, wherein the dichroic beamsplitter has a cutoff wavelength preselected to increase transmission of fluorescence signal and to decrease transmission of background noise.

10

14. The apparatus of claim 1, further comprising a modulator mechanism configured to vary the intensity of light incident on the composition without varying the intensity of light produced by the light source.

15. The apparatus of claim 1, wherein the excitation and emission optical relay structures share a multichroic beamsplitter.

16. An apparatus for measuring the polarization of light emitted from a composition, the apparatus comprising:

a stage for supporting the composition at an examination site;

an automated registration device that automatically brings successive compositions and the examination site into register for successive analysis of the compositions;

a continuous high color temperature light source;

an excitation optical relay structure having an excitation polarizer, wherein the excitation optical relay structure directs light from the light source through the excitation polarizer toward the composition;

a detector; and

an emission optical relay structure having an emission polarizer, wherein the emission optical relay structure directs light emitted from the composition through the emission polarizer toward the detector.

17. An apparatus for measuring the polarization of light emitted from a composition, the apparatus comprising:

a stage for supporting the composition at an examination site;

a continuous light source;

a time-varying light source;

an excitation optical relay structure having an excitation polarizer, wherein the excitation optical relay structure directs light through the excitation polarizer toward the composition;

a switching mechanism configured to interchangeably connect either the continuous light source or the time-varying light source to the optical relay structure;

a detector; and

an emission optical relay structure having an emission polarizer, wherein the emission optical relay structure directs light emitted from the composition through an emission polarizer toward the detector.

18. The apparatus of claim 17, wherein the continuous light source is used for steady-state polarization measurements, and the time-varying light source is used for time-resolved fluorescence polarization measurements.

19. The apparatus of claim 17, wherein the continuous light source is a continuous high color temperature light source.

20. The apparatus of claim 17, wherein the continuous light source is selected from the group consisting of a high color temperature light source, a laser, and a light emitting diode.

21. An apparatus for measuring the polarization of light emitted from a composition, the apparatus comprising:

a stage configured to support a microplate having an array of sample wells;

a light source;

an excitation optical relay structure having an excitation polarizer, wherein the excitation optical relay structure directs light through the excitation polarizer toward a composition contained in at least one of the sample wells;

a detector; and

an emission optical relay structure having an emission polarizer, wherein the emission optical relay structure directs light emitted from the composition through an emission polarizer toward the detector;

wherein the light source, detector, and excitation and emission optical relay structures are chosen so that the number of photons collected by the detector exceeds 10,000 in 100 milliseconds from a 100 picomolar fluorescein solution at pH 7.5.

22. The apparatus of claim 21, wherein the light source is selected from the group consisting of a high color temperature light source, a laser, and a light emitting diode.

23. The apparatus of claim 22, wherein the light source is a continuous high color temperature light source.

24. The apparatus of claim 21, wherein adjacent the composition light directed toward the detector by the emission optical relay structure travels anti-parallel to light directed toward the composition by the excitation optical relay structure.

25. The apparatus of claim 24, wherein the excitation and emission optical relay structures share a dichroic beamsplitter.

26. The apparatus of claim 24, wherein the dichroic beamsplitter has a cutoff wavelength preselected to increase transmission of fluorescence signal and to decrease transmission of background noise.

27. The apparatus of claim 24, wherein the excitation and emission optical relay structures share a multichroic beamsplitter.

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28. The apparatus of claim 21, wherein the fluoroscein solution has a volume less than 500 microliters.

29. The apparatus of claim 21, wherein the florescent solution has a volume less than 10 millimeters.

30. The apparatus of claim 21, the light having a cross-sectional area in the composition, wherein the area has a minimum diameter that is no larger than 2 millimeters.

20

31. An apparatus for measuring the polarization of light emitted from a composition, the apparatus comprising:

a stage for supporting the composition at an examination site;

39. The apparatus of claim 38, wherein the light source is a laser.

40. The apparatus of claim 38, wherein the light source delivers multichromatic light over a continuous range of 390 to 770 nm.

41. The apparatus of claim 38, wherein the light source is a continuous xenon arc lamp.

42. The apparatus of claim 38, wherein the excitation optical relay structure transmits sufficient light from the light source so that the number of photons collected by the detector exceeds 10,000 in 100 milliseconds from a 100 picomolar solution of pH 7.5.

43. The apparatus of claim 42, wherein the fluoroscein solution has a volume less than 500 microliters.

44. The apparatus of claim 42, wherein the fluoroscein solution has a volume less than 10 microliters.

45. A method of measuring polarization of light emitted from a composition, comprising

constantly polarizing and transmitting high temperature color light to an examination site as successive samples are automatically, serially aligned in an optical path intersecting the examination site, and

detecting polarized light emitted from each sample.

46. The method of claim 45, further comprising depositing the samples in microplate wells.


30
32

Fig. 4

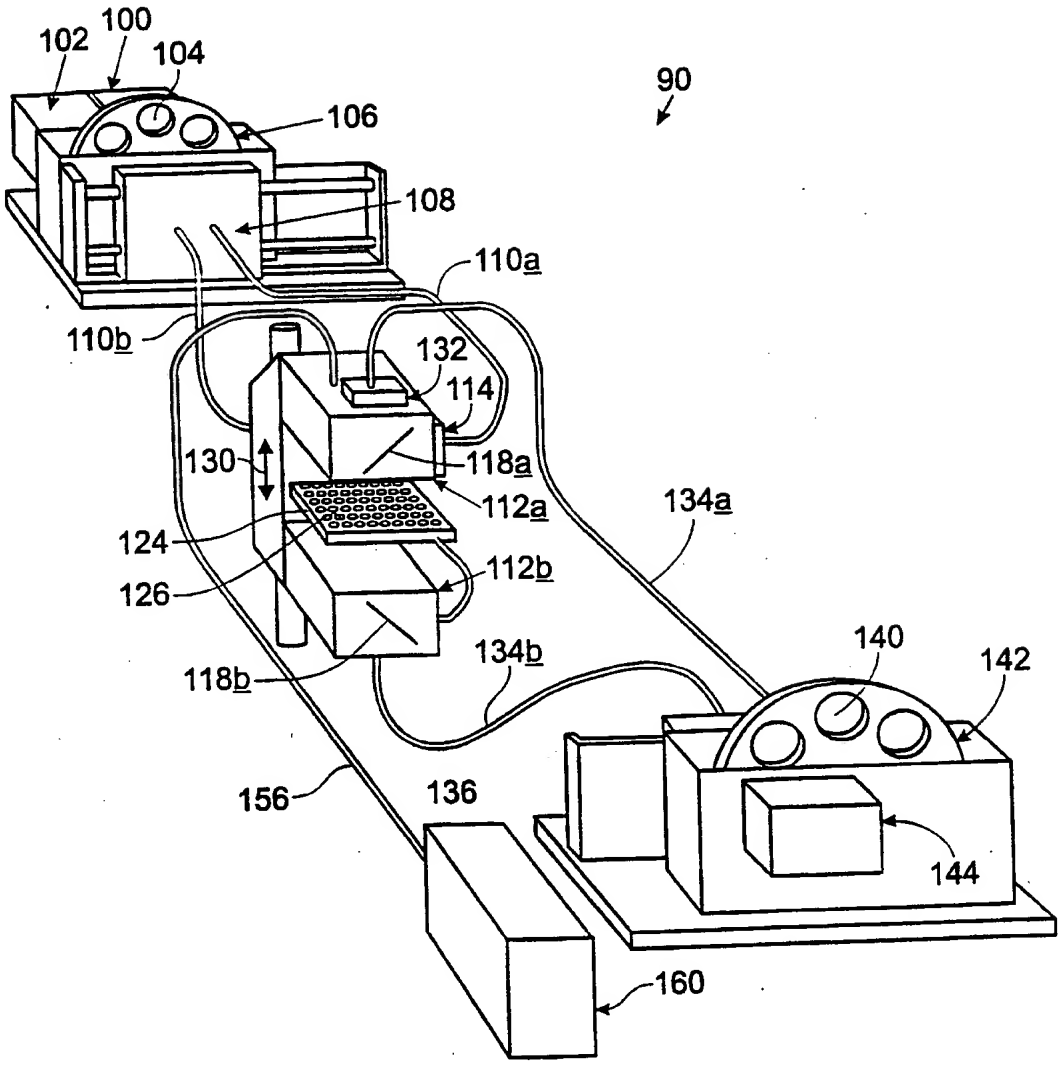
102
100
104
90
106
108
110a
110b
132
114
130
118a
112a
134a
124
126
112b
134b
140
142
118b
156
136
144
160

Fig. 7

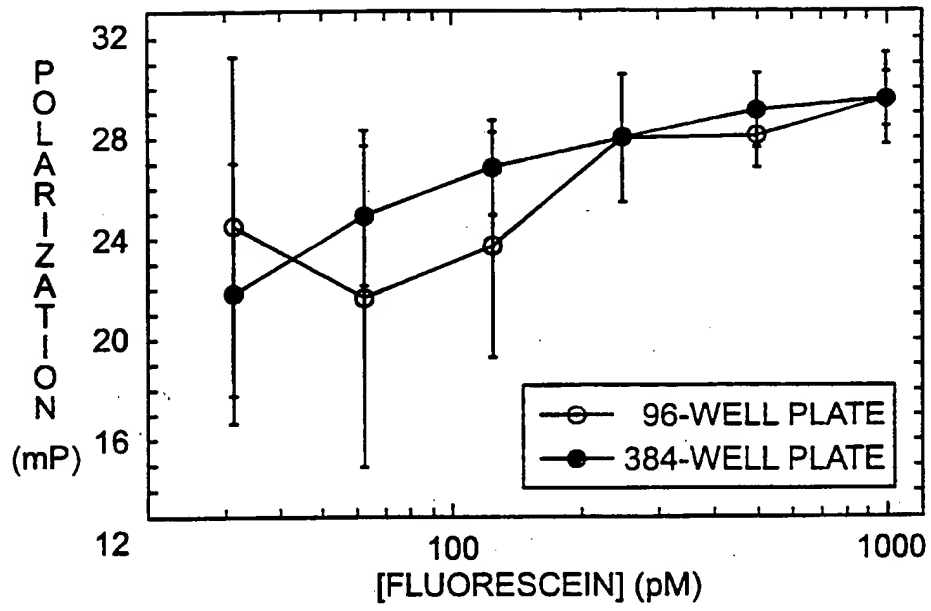
POLARIZATION (mP)
32
28
24
20
16
12
100
1000
[FLUORESCEIN] (pM)
96-WELL PLATE
384-WELL PLATE

Fig. 8

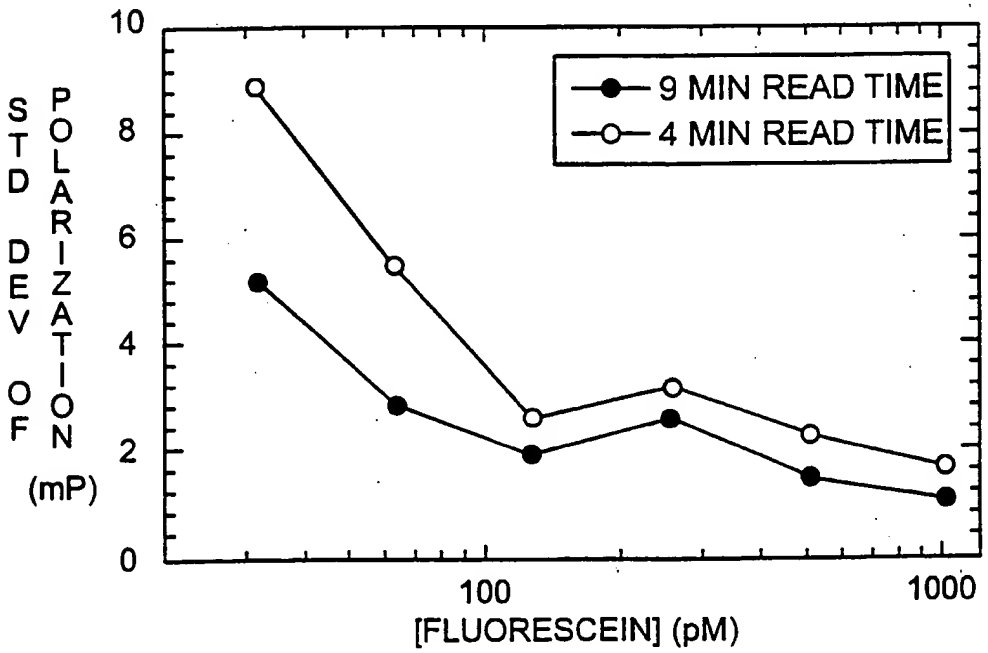
STD DEV OF POLARIZATION (mP)
10
8
6
4
2
0
100
1000
[FLUORESCEIN] (pM)
9 MIN READ TIME
4 MIN READ TIME

Fig. 9